**Prodrug: Approach to better drug delivery**

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**Abstract**

The concept of prodrug was first introduced in medicinal chemistry by Albert.

 “A prodrug is a molecule which does not have any intrinsic biological activity but which is capable during the different phases of its metabolism to generate a biologically active drug”. According to this definition and to that accepted by IUPAC, a prodrug is any compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as drugs that contain specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule.

A potent suitable prodrug should overcome the crucial paradox: it has to be lipophilic enough to cross a membrane or metabolic barrier and simultaneously it should be hydrophilic enough to fulfil solubility, bioavailability and transport criteria.

 An attractive alternative is a chemical solution that involves a prodrug approach. The prodrug approach has also been widely used to improve delivery of drugs to their site of action by modulation of physico-chemical properties that affect absorption or by targeting specific enzymes or membrane transporters. Thus, a prodrug design is a lead modification approach that is used to correct a flaw in a drug candidate and may be useful in circumventing problems associated with formulation and solubility, absorption and distribution, instability, site specificity of liberation, prolonged release and toxicity, amongst other effects.

Keyword : prodrug, enzyme, gene, pharmacodynamic, bioconversion.

**Introduction**

The prodrug concept has been used to improve undesirable properties of drugs since the late nineteenth century. Prodrugs are inactive, bio reversible derivatives of active drug molecules that must undergo transformation in vivo to release the active parent drug, which can then elicit its desired [pharmacological effect](https://www.sciencedirect.com/topics/chemistry/pharmacological-effect) in the body. In most cases, these are simple chemical derivatives that are only one or two chemical or enzymatic steps away from the active parent drug. Numerous prodrugs designed to overcome barriers to drug utilization have reached the market. Although the development of a prodrug can be very challenging, the prodrug approach represents a feasible way to improve the erratic properties of investigational drugs or drugs already on the market. This chapter introduces the rationale behind use of the prodrug approach from past to present, and also considers the possible problems that can arise in future from inadequate activation of prodrugs.[1]

# **Prodrugs in medicinal chemistry and enzyme prodrug therapies**

The traditional path to a successful drug on the market starts with identification of the disease and the proper drug target, finding the lead and the pharmacophore, and optimizing the interaction of the drug lead molecule with the target. Successful to this point, the program of drug development may still fail due to poor pharmacokinetics (PK) of the molecule. Shortcomings in PK may be associated with each of the four constituting barriers, namely Absorption, Distribution, Metabolism, and Excretion (known as ADME). In many successful examples of drugs being brought to the market, pharmacological lead is significantly optimized to improve its PK and overcome such barriers using prodrugs. By definition, prodrugs are derivatives or precursors of therapeutically active molecules, which undergo bioconversion into their active form inside the body, be it via spontaneous processes (e.g. hydrolytic degradation) or through a biocatalytic mechanism. Application of a prodrug strategy in drug delivery typically seeks to aid the drug in overcoming a barrier, physical or metaphorical, to enhance the deliverable payload of the drug. Such barriers include (but are not limited to) poor aqueous solubility – which can significantly limit utility of the drug in medicinal use; poor absorption from the gastro-intestinal tract into blood; poor rates of cell entry; etc.[2]

Prodrug bioconversion processes can arbitrarily be grouped into two categories, namely

1. prodrug degradation with resulting drug recovery, and
2. prodrug activation (Scheme 1). For the prodrug degradation class, a prodrug molecule represents a conjugate of the parent drug; bioconversion reaction involves removal of a masking group (often termed “promoiety”); and the chemical complexity of the molecule decreases. Within the prodrug activation category (also referred to as “bio precursor” category ), the prodrug undergoes a point chemical modification (e.g. cardamine-to‑carbonyl transformation ) whereby the chemical complexity of the molecule is relatively unchanged whereas therapeutic activity is markedly enhanced. Alternatively, prodrug activation involves a conjugation reaction (e.g. phosphorylation for nucleoside analogues) such that chemical complexity of the molecule is increased. Here and below, these two classes of prodrug bioconversion are denoted as “drug recovery” and “prodrug activation”, respectively.[2]

Design strategy for a prodrug first of all depends on the structural features of the parent drug molecule and specifically the availability of appropriate chemical functionalities that can be used to mask pharmacodynamic activity of the drug through e.g. an attachment of the modifying group. The second, equally important consideration pertains to the bioconversion mechanisms of drug release. This process may be spontaneous, yet in the overall majority of cases, drug synthesis (via recovery or activation) is carried out through an enzymatic process. Conventional prodrugs in medicinal chemistry are typically designed to achieve a quantitative recovery of the drug and this is the prime objective. In these applications, there typically is little to no consideration of the drug distribution within the body, and the enzyme to perform the bioconversion may be distributed throughout the body (e.g. esterase, phosphoesterases). In some instances, specific enzyme may be predominantly expressed within a particular organ such as in the liver and bioconversion of a prodrug is achieved predominantly therein (e.g. first step of capecitabine bioconversion performed by carboxylesterases and HepDirect prodrugs for activation by cytochromes). Design of these prodrugs for general medicinal use is reviewed rather well and in the presentation below it is considered only briefly to provide a proper context, scientific as well as historical.[2]

The main focus of this review is the design of prodrugs for an advanced drug delivery opportunity termed Enzyme Prodrug Therapy (EPT). For this, a cunning sub-class of prodrugs is designed such that bioconversion is performed by a specifically nominated enzyme placed in a particular location in the body. Through this, recovery or activation of the drug is achieved only at the location of the enzyme. In contrast to the general medicinal prodrugs, in the case of EPT, quantitative drug recovery is less important and the prime goal is to achieve a site-specific drug recovery. Localization of the enzyme at the desired site can be accomplished using a number of ways, with varied degrees of success and progression from lab to clinic. Historically, antibody-directed enzyme-prodrug therapy (ADEPT) marked the earliest broadly-recognized success of EPT . This approach to EPT is injection-based whereby the enzyme is conjugated to an antibody and the latter facilitates anchorage of the enzyme at the site of action. Examples of ADEPT typically rely on extracellular prodrug bioconversion through drug recovery mechanisms. Early successes were also documented using encapsulated enzymes surgically placed at the site of resected tumour for post-operative chemotherapy . This mode of EPT has recently seen a considerable revival of interest. The mode of EPT with currently the highest number of ongoing clinical trials is that of gene-directed EPT (GDEPT), also known as “suicide gene therapy” . In this case, the enzyme for prodrug conversion is expressed by the cells upon transduction of the latter and this is most successfully accomplished using viral vectors. GDEPT examples almost exclusively rely on intracellular activation of the administered prodrugs.[2]

# **Strategies for Enzyme/Prodrug Cancer Therapy**

Enzyme-activating prodrug therapy is a two-step approach. In the first step, a drug-activating enzyme is targeted and expressed in tumours. In the second step, a nontoxic prodrug, a substrate of the exogenous enzyme that is now expressed in tumours, is administered systemically. The net gain is that a systemically administered prodrug can be converted to high local concentration of an active anticancer drug in tumours. To be clinically successful, both enzymes and prodrugs should meet certain requirements for this strategy. The enzymes should be either of nonhuman origin or human protein that is absent or expressed only at low concentrations in normal tissues. The protein must achieve sufficient expression in the tumours and have high catalytic activity. The prodrug should be a good substrate for the expressed enzyme in tumours but not be activated by endogenous enzyme in nontumor tissues. It must be able to cross the tumour cell membrane for intracellular activation, and the cytotoxicity differential between the prodrug and its corresponding active drug should be as high as possible. In addition, the half-life of active drug should be long enough to induce a bystander effect but short enough to avoid the drug leaking out into the systemic circulation.[3]

In the choice of the appropriate enzyme/prodrug combination, priority should be given to the enzyme. From past experience, it is likely that suitable prodrugs can be designed for almost any enzyme substrate specificity (Connors, [**1995**](https://onlinelibrary.wiley.com/doi/full/10.1002/1097-4652%282001%299999%3A9999%3C%3A%3AAID-JCP1060%3E3.0.CO%3B2-H#bib36)). Expression of the enzyme itself should not lead to cytotoxic effects; the bystander effect required (see the Bystander Effect section) would not be achieved if the cells were killed by the action of the enzyme alone. The reaction pathway should also be different from any endogenous enzyme, in order to avoid cytotoxic activation of the prodrug in normal tissues. This is the main drawback of utilising proteins of human origin, which, on the other hand, have the potential advantage of avoiding complications of acquired immunity, in particular after prolonged administration or prolonged protein expression. The selected prodrug should be freely diffusible throughout the tumour (possibly a neutral species), chemically stable under physiological conditions and have suitable pharmacological and pharmacokinetic properties. For significant therapeutic gain, the released drug should be at least a 100-fold more toxic than the prodrug. The toxic agent should also have a half-life that allows diffusion to the surrounding transfected cells (bystander effect), but ensures that any drug escaping into the circulation will be inactive. Moreover, the induced cytotoxicity should be cell cycle phase- or proliferation-independent, to kill a wide range of tumour cell populations.[4]

## Approaches to Deliver Prodrug-activating Enzymes into Tumor Cells or Tissues

ADEPT is a strategy in which a tumor-associated monoclonal antibody is linked to a drug-activating enzyme to create a systemically administered conjugate that only targets tumor tissues. Nontoxic prodrug is then administrated systemically and is converted by the pretargeted enzyme localized on the tumor surface into a toxic drug, resulting in cytotoxic effects in tumor cells . The ideal drugs for ADEPT are small molecules that can diffuse within the tumor tissues, including both antigen-positive and antigen-negative tumor cells, and cause a bystander effect . When ADEPT is applied clinically, the interval between enzyme and prodrug administrations should be optimized so that the conjugate is only accumulated in tumours rather than in blood and normal tissues, to avoid systemic toxicity. ADEPT has been used to deliver many drug-activating enzyme genes to tumours in vitro and in vivo.

 The target antigen should be either expressed on the tumor cell membrane or secreted into the extracellular matrix of the tumor , and the use of a high affinity monoclonal antibody is essential . The enzyme should be able to exert its optimal activity at a pH close to that of the tumor extracellular fluid. Because antibody-enzyme conjugate may be immunogenic, circulating host anticonjugate antibodies may interfere with treatment. Therefore, the drug chosen should be dose dependent and cell cycle independent . Ideally, the enzyme system should not have a human homologue to avoid prodrug activation outside the tumor site .

Because the interval between enzyme and prodrug administrations is important for ADEPT, some studies were performed to explore the optimal interval in animals. Linking the enzyme CPG2 to the anti-CEA antibody A5B7, a rapid clearance of conjugate from the circulation was demonstrated, allowing the prodrug CMDA to be safely given 48 h or 72 h after antibody-enzyme administration . In human subjects, 7 days were needed for adequate clearance of antibody-enzyme conjugate from the plasma before the prodrug may be administrated safely, to avoid activation of prodrug in plasma and subsequent systemic toxicity . Recently, this CMDA/CPG2 prodrug/enzyme system has been used in a Phase I clinical trial of 10 patients with colorectal carcinoma . The bacterial enzyme CPG2 was conjugated to the F(ab′)2 fragment of murine A5B7 monoclonal Ab, and a galactosylated second clearing Ab against CPG2 was also used to lower levels of conjugate in the circulation and other nontumor tissues. The aim of the trial was to measure plasma levels of the prodrug CMDA and active drug CJS11, a bifunctional alkylating agent, released from prodrug by the action of CPG2 localized in tumors. CPG2 activity was found in metastatic tumor biopsies, but not found in normal tissues, after applying the clearing agent. The rapid appearance of the active drug with half-life of 36 ± 14 min in plasma was observed in this system . This initial example provides promise for the use of ADEPT to improve the selectivity of current therapy for solid tumors.

Like GDEPT and VDEPT, there are many clinical limitations associated with ADEPT. In poorly vascularized tumors, delivery of the large conjugate is restricted, and it is not possible to deliver antibody/enzyme conjugate to all of the tumor cells . Because the enzyme level is low, it is very difficult to generate adequate quantities of active drug to reach the lethal concentration. Furthermore, the binding of the conjugate to the cell surface is limited by antigen heterogeneity. Other drawbacks of ADEPT include cost and difficulties with development and purification of antibodies, immunogenicity of antibodies, accessibility of tumor to the enzyme/antibody conjugate, and the conversion of prodrugs in nontumor tissues . The main problem with ADEPT is the immunogenicity of the antibody-enzyme conjugate, which limits multiple cycles of its application . To solve this problem, several solutions have been tried, including the use of humanized proteins and concomitant administration of immunosuppression .[4]

Because of the problems mentioned above, many ways have been tried to improve ADEPT. The first way to improve ADEPT is to use a three-phase system to speed up the removal of enzymes from the circulation without affecting the enzyme activity in tumour tissues . In this approach, a galactosylated anticonjugate antibody was applied after the administration of conjugate and prodrug as a clearing agent that reacted with the conjugate in the plasma, thus decreasing its blood levels, but retaining enzymatic activity in tumours . A second way to improve ADEPT is to use a conjugate containing an enzyme and a partial fragment of antibody, which would be cleared more rapidly from the circulation, with the prodrug given earlier, whereas the enzyme level within the tumour is at the peak concentrations . The third way to improve ADEPT is to combine ADEPT with an antivascular agent, a drug that selectively inhibits tumour blood flow and causes extensive necrosis. In a study of nude mice bearing a colorectal tumour xenograft, a conjugate containing the bacterial CPG2 and the F(ab′)2 fragment of anti-CEA antibody to activate the prodrug CMDA was combined with the antivascular agent 5, 6-dimethylxanthenone-4-acetic acid at 20 h post conjugate injection, resulting in killing a larger part of tumour, doubling the concentration of antibody-enzyme conjugate retained in tumour, and significantly prolonging the tumour growth inhibition caused by ADEPT alone . Furthermore, 5, 6-dimethylxanthenone-4-acetic acid also increased prodrug retention within the tumour by 16-fold. The fourth way to improve ADEPT is to use mutant form(s) of human enzymes to avoid systemic toxicity caused by the use of wild-type human enzyme(s) and decrease immune responses caused by the use of nonhuman enzyme(s) . A mutant form of human CPA conjugated to a tumor-associated antibody was effective to activate several prodrugs, including thymidylate synthase inhibitors GW 1031 and GW 1843 and the dihydrofolate reductase inhibitor MTX, whereas all these prodrugs were not efficient substrates for endogenous CPA. The use of mutant human enzymes may provide less immunogenicity than nonendogenous enzymes and less systemic toxicity than endogenous enzymes . The final way to improve ADEPT is to use recombinant DNA technology to produce a fusion protein with defined characteristics and to avoid additional antibody purification steps, which may cause reduced enzymatic activity or decreased antibody binding of the conjugate . The combination of this fusion protein with a doxorubicin prodrug resulted in superior growth inhibition when compared with prodrug alone . Recently, an expression plasmid for the production of a fusion protein containing the single-chain Fv anti-CD20 mouse monoclonal antibody and human lysosomal enzyme β-glucuronidase was found to bind CD20-expressing lymphoma cells in a specific manner and was able to activate the prodrug N-[4-daunorubicin-N-carbonyl (poxymethyl)phenyl]O-b-glucuronyl carbamate at a rate similar to that of purified human β-glucuronidase.[5]

# **Bacterial-directed enzyme prodrug therapy**

The selectivity of bacterial growth within tumours relates to a tissue phenotype that distinguishes tumour tissue from healthy tissue. Ironically, the microenvironment of the tumour which protects it from most anticancer treatments represents the ‘Achilles heel’ that sensitises it to bacterial anticancer agents. It is well documented that different bacteria preferentially accumulate in various experimental tumours. For example Salmonella strain VNP20009 has demonstrated ratios of tumour to normal tissue of 300–25,000:1 . Various theories have been proposed in order to explain such observations. The primary factors that underpin this specificity are direct or indirect results of tumour growth processes ultimately resulting in zones of necrosis. In order for tumours to grow and develop, they require new blood vessels to be formed, a process known as neo angiogenesis. It is a hallmark of cancer and essential for the continued supply of oxygen and nutrients to the tumour . Once the tumour radius reaches a critical mass, oxygen can no longer adequately reach the inner layers of the tumour, and the cells become gradually hypoxic. In the hypoxic zone, the low-oxygen partial pressure induces further angiogenesis. These newly formed vessels are abnormal in structure and function and create physiological barriers to the delivery of therapeutic agents, and immune cells . One of the exploitable features of their abnormality is that they consist of pores of various sizes ranging from 200 nm to 2 μm (depending on the tumour)[6]. This potentially allows micro-organisms such as bacteria to egress from the vasculature and lodge locally within the tumour mass. Necrotic regions are areas of dead cells usually but not exclusively found in the middle of the tumour mass. Such zones are permissive for bacterial growth as they would be expected to provide protection from the immune system and sufficient nutrients (e.g. purines) from the dead tumour cells. Indeed, surgeons have reported anecdotally, some tumours (usually large with extensive necrotic regions) producing a decaying odour upon surgical resection, most likely originating from infecting microorganisms.[7]

The exact location of bacterial proliferation within the tumour may vary between species. A recent 3D imaging study indicated the growth of anaerobic bifidobacterial as multiple clusters within non-viable tumour regions . Evidence by Forbes et al. demonstrated that salmonellae proliferated within the necrotic areas of model tumours. Such an observation implies that their use is limited to large tumours. However, this contradicts earlier data published by and recent data by , which demonstrate Salmonella proliferation in both normoxic and hypoxic areas. Such a capacity is preferred in a clinical context. An ideal bacterial anticancer agent should target to and proliferate within micro metastatic tumours which naturally lack necrotic regions. For example, Escherichia coli K12 MG1655 and HJ1020 tagged with light emitting genes have been shown to target very small tumours as well as large ones and even anaerobic Bifidobacterium breve has displayed a similar capacity.[8]

Bacteria offer several advantages as vectors for cancer gene therapy. For example, bacteria are easily manipulated to generate exogenous products of therapeutic relevance, to improve their tumour selectivity, or to express prodrug activating enzymes and reporter proteins for visual confirmation of treatment location and therapeutic outcome. Different types of bacteria have different mechanisms of achieving tumour specificity. Obligate anaerobes such as Gram-positive Clostridium species can form spores that are only able to germinate inside the anoxic regions of tumours . In contrast, facultative anaerobes, such as Gram-negative Salmonella and Escherichia, accumulate inside tumours for several reasons: protection from the immune system, positive chemotaxis towards resources inside the tumour micro-environment and entrapment in the chaotic vasculature of tumours. Bacteria offer one additional key advantage over their viral vector counterparts – bacterial infection during cancer therapy can readily be controlled by antibiotics.[9]

Tumors require effective blood supply for their growth. The inhibition of angiogenesis is a promising strategy for treating oncologic patients. Despite of many endogenous inhibitors of angiogenesis being found, clinical evaluation were prevented by the need of high doses, production limits and the relative instability of the proper recombinant proteins. [10] Antiangiogenic therapy is specifically directed against microvascular endothelial cells created in the tumor location. Specific antiangiogenic therapy has low or none toxicity at all, does not demand the entrance of therapeutic agents into tumor cells and does not pass the hematoencephalic barrier. It controls the tumor growth, independent of the cell type of the tumor and does not induce acquired drug resistance. [11] The supplementation of genes coding antiangiogenic proteins is a promising procedure avoiding obstacles connected to systemic application of medicaments. Therapeutic genes coding antiangiogenic substances can be distributed to patients by numerous carrier systems, for example by recombinant adenoviruses or liposomes. [12] Antiangiogenic gene therapy can be carried out as a systemic or local treatment. Scientists still cannot agree on the best means of application. Local (intratumor) application is joined with a strong “bystander effect”, increasing the antagonistic activity of introduced genes and should not be connected with potential side effects of systemic therapy. [13] On the other hand, systemic application of genes coding antiangiogenic factors enables a long-lasting elevation of endostatins in blood. [14]

Conclusion

This review aimed to present the enzymes used to achieve bioconversion of prodrugs as well as the considerations related to the synthesis and utility of prodrugs specific to these enzymes, with a particular emphasis on enzyme-prodrug therapies. Current state of the art in prodrug design already presents this field as highly successful, so many of marketed therapeutics being prodrugs. In contrast, from advent several decades ago, EPT has revealed minor success. There are advantages of ADEPT when compared to conventional therapy: there is increased selectivity for malignant cells which takes advantage of the specificity of the Ab; internalisation into tumour cells of the Ab-enzyme conjugate is not required; there is an amplification effect since each enzyme molecule is able to cleave a large number of prodrug molecules; in the majority of examples described here, the released active drug is of low molecular weight which enables it to.

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